Studies on the Duck Egg White Proteins, especially the Ovalbumin

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Introduction

The ducks spend most of their time on or near water, though they are semiaquatic. In taxonomy they belong to the Anseriformes, differing from the chicken and the quail belonging to the Galliformes. In the earlier days, Bain and Deutsch[1] performed electrophoretic studies on the egg whites of different birds belonging to various species of the Galliformes, Anseriformes and the Columbiformes, and described that the mobilities of ovalbumins of the Anseriformes species (mullard duck and goose) are larger than those of the Galliformes species (chicken and turkey etc., ...) at *pH* 8.6 and ionic strength 0.1. Similar relationship was also fixed by another researcher at *pH* 5.2 and ionic strength 0.02[2].

The electrophoretic mobility depends upon the isoelectric point of the protein, hence the isoelectric point of the duck ovalbumin is inferred to be lower than that of the chicken's one.

Feeney et al.[3] ascertained that the specific activities of lysozymes from the duck and the chicken egg whites were the same, though the lysozyme content in the former was smaller than that in the latter, and moreover, conalbumin content in the duck egg white was remarkably small. In comparison with the sulfhydryl groups amount in the chicken ovalbumin, the smallness of the amount in the duck ovalbumin was confirmed. In a study of the deterioration of the duck and the chicken shell eggs, Rhodes and Feeney[4] presumed that the smallness of sulfhydryl group and lysozyme content in the duck white might be responsible for the high resistance to the deterioration.

Fothergill and Perrie[5] inferred a surprisingly large difference in the structure between duck and chicken ovalbumins which are serologically so similar. As described above, the investigations on the duck egg white proteins have been very partially conducted. Judging from the experimental results of other workers and the general properties of duck white estimated by the authors, relative content, chemical composition and structure of the constituent proteins in duck white are supposed to be differing from those in chicken egg white. The authors are going to progressively examine the chemical characteristics of each constitutional protein.

In the present paper, for the separation of duck's egg white proteins, CM-cellulose chromatography and the gradient extraction with salt were conducted, and chemical constitutions

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of the ovalbumin were particularly examined.

Experimental procedures

Materials—The duck eggs were secured from the farm feeding the domestic birds near the University of Kagoshima, within 24 hours after being laid. After the procurement the eggs were weighed individually and were broken. The egg white was separated as completely as possible from the yolk with the aid of injector, and the weight of the white, yolk and shell were measured respectively. The chalazae was removed with a pair of tweezers and the white was blended with a homogenizer at a fixed speed. The homogenized egg white was used as the sample for various measurements and analyses.

Examination of general chemical properties—The specific gravity of the egg white was measured with the Ostwald picnometer. The determination of nitrogen was carried out by the semimicro Kjeldahl method. The electric resistance was measured with the Kohlrausch bridge in the water at 15°*C* and the specific conductance was calculated from the resistance value.

The determination of total neutral sugar—The total neutral sugar was determined by the phenol-sulfuric acid method proposed by Dubois et al.[6] and its colour intensity was measured at 490 mμ with Shimazu-Bausch Lomb Spectronic 20 spectrophotometer.

General separation of egg white by CM-cellulose column chromatography—The separation of the whole egg white on CM-cellulose was performed by the method as described particularly in the previous report,[7] and the gradient elution due to the continuous *pH* rise was adapted for chromatography. Twenty *ml.* of 1.36% sample in 0.025*M* acetate buffer was applied on CM-cellulose column (1.8 × 20cm.). The eluate *pH* was measured at each fractionated tube (5*ml.*) and subsequently the absorbancy at 280 *m*μ was measured with the Hitachi 101 spectrophotometer, from which value the protein content was calculated. The purified ovalbumin from duck egg white was employed as the standard for the protein estimation (shown in Fig. 7).

Separation by gradient extraction with ammonium sulfate—The full saturation was achieved by adding the excessive ammonium sulfate to the dilution (4.43 %) 4*ml.* of the homogenized egg white, and small amount of Celite (1 *g.*) was added on it. The precipitated proteins and the supernatant were poured slowly on the Celite layer of the extracting apparatus as shown on the previous report.[7] The extraction of the precipitated proteins and the measurement of the concentrations of ammonium sulfate solution were performed as described previously. The effluent was collected in 5 *ml*. fractions with the electric fraction collector (Drop count type of *Tōyoo Kagaku Co. Ltd.).* The protein and sugar contents were determined at each fraction.

Preparation of ovalbumin—Ovalbumin was separated from the homogenized egg white by the salting-out with ammonium sulfate followed by the recrystallization repeated several times. However, ovalbumin separated by this procedure was not refined, enough; including a minor other protein. Therefore, this ovalbumin was chromatographed on a column of CM-cellulose (2.3 × 25*cm.*) and the fractions corresponding to the ovalbumin peak were collected, and then dialyzed against 15 % polyethylene glycol for concentrating. After dialyzing the solution against the water, and subsequently 0.1 *M* acetate buffer (*pH* 4.50), the ovalbumin

solution (0.78%, 4 *ml.*) was applied on Sephadex G-100 column of 2.2*cm.* width, 52 *cm.* length and collected in 5 *ml.* fractions. The protein and sugar content in each fraction were separately determined. The combination of ovalbumin fractions obtained by multiple gel filtrations was dialyzed against the water, and the ovalbumin aqueous solution was used as the sample for sugar and amino acid analyses.

Identification of neutral sugar in ovalbumin—The analysis of neutral sugar was done qualitatively. The purified ovalbumin (400 *mg.*) was hydrolyzed with 40 *ml.* of 1 *N* H_2SO_4 in a sealed tube for six hours at 100°C and the hydrolysate was filtered and diluted to 500 *ml.* The solution was treated with the Amberlite IR-120 column (2.1 × 42 *cm.*), followed by the Amberlite IR-4B column (2.1 × 37 *cm.*), and concentrated under reduced pressure. The solution was employed for paper chromatography, using buthanol-acetic acid-water (12:3:5, *v/v*) as solvent. Saturated $AgNO_3$-aceton (0.5:100, *v/v*) and 0.5 *N* NaOH in ethanol were used for developing the colour.

Identification and determination of hexosamine in ovalbumin—The identification and determination of hexosamine in the ovalbumin were performed according to the Pearson's procedure[8] and the Elson-Morgan method modified by Boas[9]. Ten *ml.* of 8 *N* HCl was added on the ovalbumin aqueous solution (0.89 %, 10 *ml.*) in the pyrex tube and the mixture was hydrolyzed in a sealed tube for six hours at 100°C. The hydrolysate was filtered through the glass filter and concentrated to a small volume under reduced pressure at 45°C. It was evaporated at room temperature in a vacuum desicator over NaOH particles and **kept** for about ten days. The dried hydrolysate was dissolved and diluted to be 50 *ml.* with 0.3 *N* HCl. This solution (2 *ml.*) was applied on a column (0.6 × 39 *cm.*) of Amberlite CG-120 for chromatography. Fraction volume was adjusted to be 1 *ml.* and 0.3 *N* HCl was used as the eluting agent. To each fraction, 1 *ml.* of water and 1 *ml.* of acetylaceton reagent were added. The tubes were stoppered and suspended in a water bath at 90°C for 45 minutes. After cooling, 2.5 *ml.* of ethyl alcohol was added; after mixing, 1 *ml.* of Ehrlich's reagent (2.67 % solution of *p*-dimethylaminobenzaldehyde in 1:1 mixture of ethyl alcohol and concentrated HCl) was added. After careful shaking, the optical densities were read at 530 *mμ* on a Hitachi 101 spectrophotometer. For the identification and determination of the sample hexosamine, a known amount of authentic galactosamine and glucosamine were separately chromatographed under the same condition.

Amino acid analysis of the ovalbumin—Five *ml.* of 12 *N* HCl (special grade) was added on the ovalbumin aqueous solution (0.89 %, 5 *ml.*) in the pyrex test tube and after careful shaking, the air was removed by the suction. After introducing the nitrogen gas into the tube, the tube was sealed. The hydrolysis was carried out for 24, 30 and 48 hours at 110°C respectively. The hydrolysate was filtered through the glass filter, and was concentrated to be a small volume under reduced pressure and diluted to 100 *ml.* volume with the citrate buffer (0.2 *N* as Na ion, *pH*, 2.2). This solution (0.5 or 1.0 *ml.*) was employed for the analysis of amino acids excepting tryptophan by using *Yanagimoto LC-5S* type amino acid analyzer. Tryptophan was determined by the method due to the ultra violet absorption.[10]

Results and discussion

General chemical properties—The general chemical properties of the duck egg white compared with those of the chicken egg white are as shown in Table I.

Feeney et al.[3] described that the white and yolk indices in twenty five different species

Table I. General chemical properties of the duck egg white compared with those of the chicken's one

	Duck	Chicken (White leghorn)
Weight of entire egg (*g.*)	65.4～84.9 (20)	52.4～67.2 (10)
White/Yolk (*W/W*)	1.5～2.0 (20) Av. 1.7	1.9～2.3 (10) Av. 2.1
White/Egg ×100 (%)	52.2～57.9 (20)	58.4～62.5 (10)
Solids in egg white (%)	10.8～12.7 (8) Av. 12.1	10.5～11.5 (5) Av. 11.1
Water in egg white (%)	89.2～87.3 (8) Av. 87.9	89.5～88.5 (5) Av. 88.9
Specific gravity at 30°C	1.034～1.037 (12)	1.039～1.052 (5)
Nitrogen in dry matter (%)	12.9～13.4 (5) Av. 13.1	13.8～14.1 (5) Av. 14.0
Sugar-protein ratio	7.67×10^{-2} (8)	7.18×10^{-2} (5)
Sugar-protein ratio after dialysis against carbonate* (*pH* 9.80)	3.92×10^{-2}	3.71×10^{-2}
pH	8.00～8.30 (40)	7.90～8.30 (20)
Electric resistance at 15°*C*	140～135 *ohm*	161～154 *ohm*
Specific conductance at 15°*C*	$7.19\sim7.41\times10^{-3}$ *mho*	$6.23\sim6.51\times10^{-3}$ *mho*
Sp. conductance in C=4.9%, at 15°*C*	3.46×10^{-3} *mho*	3.25×10^{-3} *mho*
Sp. conductance after dialysis against H_2O, C=5.0%	4.72×10^{-4} *mho*	5.31×10^{-4} *mho*

Numbers in parentheses are the egg used for measuring.
Av.; Average value * 0.05 *M* carbonate buffer

resembled with one another. Similary, the examination of the author showed that there is no noticeable difference in the white and yolk index, solid content in egg white between the duck and chicken eggs. Comparing with the chicken egg white, it was ascertained that the specific gravity and nitrogen content of the duck egg white are smaller, while the sugar-protein ratio is higher. The ratio of the combined sugar to the protein was measured after removing the low molecular substances by dialysis. The carbonate buffer (*pH* 9.80) producing no protein precipitates was used for the dialysis. The ratio was amounting to 3.92×10^{-2} in the duck and 3.71×10^{-2} in the chicken. This result suggests that the ovomucoid content in the former is more than that in the latter or that the other protein containing much combined sugar exists in the former, which is consistent with the fact of its having low nitrogen content. The specific conductance of the duck white was higher than that of the chicken in native state and after adjusting the concentration to be equal mutually, the similar relation was observed between the two egg whites. However, the coductivities of both egg white after dialyzing against the water showed the quite reverse relation, when compared with the result mentioned above. Those suggest the existence of a relatively small amount of charged groups in the duck egg white proteins.

Separation of egg white by CM-cellulose column chromatography—The elution diagram of the duck egg white proteins separated by CM-cellulose was shown in Fig. 1. The elution diagram reveals eight components. Those components were marked A, B, C. . . X and G in the

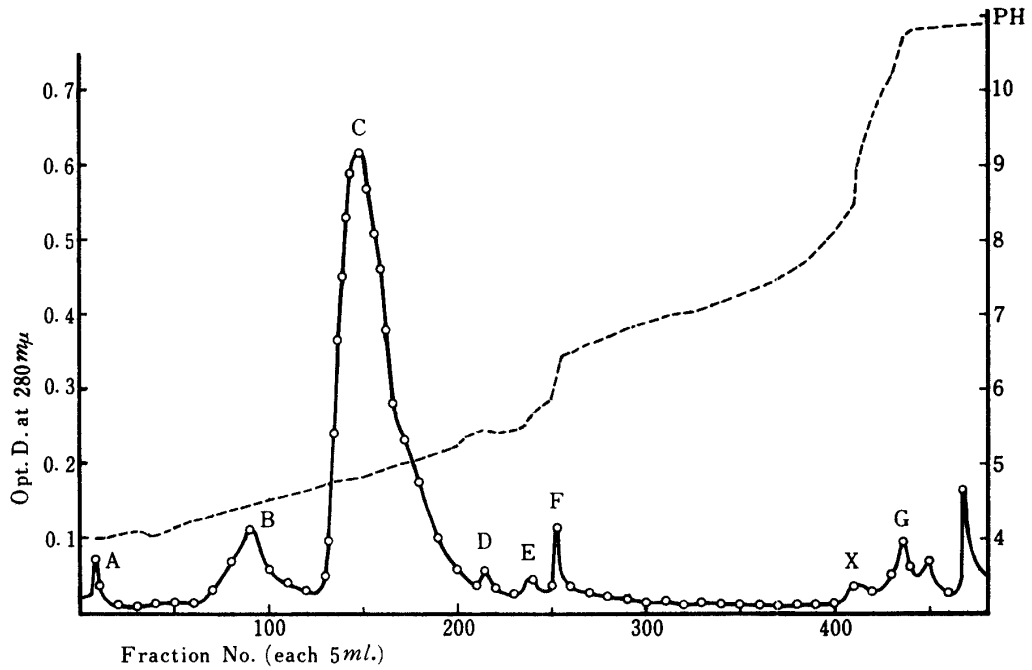

Fig. 1. Separation of the duck egg white proteins by CM-cellulose column chromatography

—○—○— Protein, ——————— *pH*

order of their elution. The final peak unmarked was considered to be the column-denatured protein. The separation pattern is a slightly similar to those of the chicken and quail egg white as shown in the previous report [7] and different from that of the emu egg white.[11] The relative proportion of each component in the egg white and the respective *pH* of the eluate at each peak were represented in Table II. Since the measurements of the isoelectric point of each constituent protein in duck egg white have not been done, each component shown in the figure can not be correctly identified. However, it is not impossible for us to estimate the separated component from the order of elution and the *pH* values of the eluate at peak, referring to the literature.[7][12][13] The authors inferred that component A is the protein being

Table II. Relative protein contents and *pH* values for elution of each component of the duck and chicken egg whites separated by CM-cellulose column chromatography

Component		A	B	C	D	E	F	G	X	others	Total
Duck	Protein (%)	1.1	9.1	71.1	1.6	1.2	1.9	5.1	—	8.8	100
	pH of eluate at peak	4.0	4.45	4.80	5.47	5.60	6.20	10.70			
Chicken	Protein (%)	6.5	2.8	44.0	5.0	3.7	14.1	14.3	2.5	7.1	100
	pH of eluate at peak	4.0	4.52	4.93	5.32	5.68	5.95	11.02	9.30		

A: Protein being anionic at *pH* 4.0, B: Ovomucoid fraction, C: Ovalbumin fraction
D, E: Ovoglobulin fraction, F: Conalbumin fraction, G: Lysozyme fraction

anionic at *pH* 4.0 (I.E.P.<4.0), B ovomucoid, C conalbumin, D globulin (G_3), E globulin (G_2), F conalbumin and G lysozyme (G_1). Comparing with the eluate *pH* at each peak of the chicken egg white proteins, values of the duck's ovomucoid and ovalbumin are lower respectively. This suggests that each isoelectric point of those proteins is lower in the duck than in the chicken respectively. The eluate *pH* of lysozyme is slightly small, but not sure, because lysozyme pattern is not so sharp as those of the chicken and quail. Comparing with the chicken egg white proteins, in the duck egg white proteins, much of them are ovomucoid and ovalbumin, while the components corresponding to conalbumin and lysozyme are small in quantity. Especially, large amount of ovalbumin is noticeable.

Separation of egg white by gradient extraction with ammonium sulfate—Separation pattern of the egg white proteins by the gradient extraction method with ammonium sulfate is as shown in Fig. 2. The protein and sugar content in each component are as represented in Table III. Sugar patterns in Fig. 2 reveal the distribution of the sugar combined with the protein, excepting the component A.

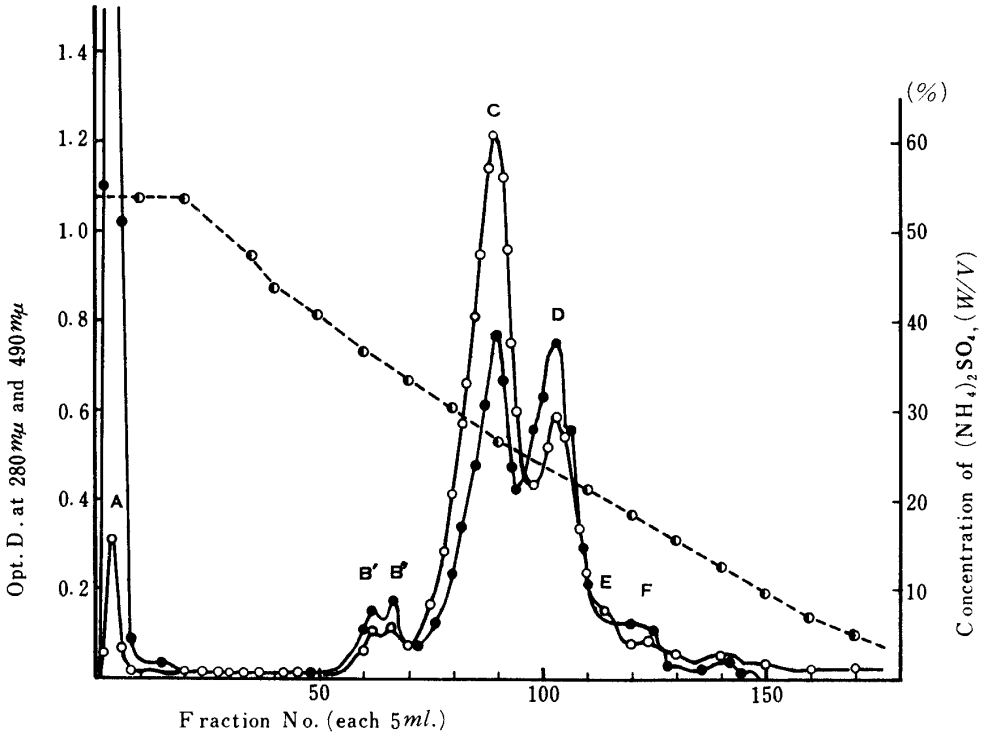

Fig. 2. Separation of the duck egg white proteins by the gradient extraction with salt

—○——○— Protein, —●——●— Neutral sugar,
---◐------◐--- Concentration of ammonium sulfate

In general, as the classification of protein is based on the solubility, judging from this standpoint it is to be inferred that each of the component A and B is chiefly glycoprotein (ovomucoid), C ovalbumin and conalbumin mixture, and D, E, F ovoglobulin respectively. Component A is not to be precipitated even by full saturation with salt. As free sugar and amino

Table III. Protein and sugar contents of each component of the duck and chicken egg whites separated by gradient extraction with ammonium sulfate

	Component	A	B′	B″	C	D	E	F	Others	Total	Sample
Duck	Concn. of salt (*W/V*, %)	53.5	38.5 ≀ 35.5	35.0 ≀ 33.2	33.0 ≀ 25.0	24.5 ≀ 21.3	21.0 ≀ 19.0	18.5 ≀ 15.5			
	Protein (*mg.*)	6.10	4.05	3.70	100.45	37.66	7.39	6.08	9.41	174.8	177.4
	Protein index	3.49	2.31	2.12	57.45	21.55	4.22	3.48	5.38	100	
	Sugar (*mg.*)	10.02	0.31	0.22	3.30	3.20	0.41	0.33	0.26	18.05	
	S-P ratio($\cdot 10^{-2}$)	164.3	7.65	5.95	3.29	8.50	5.55	5.48	2.78	10.32	

	Component	A	B	C	D	E	F	Others	Total	Sample
Chicken	Concn. of salt (*W/V*, %)	52.6	40.5 ≀ 34.0	33.8 ≀ 23.4	23.0 ≀ 19.5	19.0 ≀ 12.0	12.0 ≀ 3.5			
	Protein (*mg.*)	6.59	6.44	161.35	5.73	8.91	3.39	1.80	194.2	194.1
	Protein index	3.40	3.31	83.08	2.95	4.59	1.74	0.93	100	
	Sugar (*mg.*)	6.16	0.52	5.46	0.12	0.12	0	0	12.39	
	S-P ratio($\cdot 10^{-2}$)	93.5	8.14	3.38	2.10	1.36	0	0	6.38	

acid are mixed in this fraction and fluctuation of the sugar-protein ratio is conceivable, the value of that component is meaningless.

Comparing with the chicken proteins, in the duck egg white, glycoprotein (or ovomucoid) and globulin are relatively large, especially globulin being remarkably large. The sugar-protein ratio of the globulin was higher than those of the chicken and quail globulins, amounting to 8.50×10^{-2} in the former, 2.10×10^{-2} and 2.12×10^{-2} in the latter,[7] respectively.

It remains as a very interesting matter which should be left to further investigations. A new type protein, which elutes at the same *pH* value as ovomucoid on CM-cellulose column chromatography in globulin fractions, was ascertained by authors, the details of which will be published later. The component corresponding to the duck ovalbumin and conalbumin is smaller in quantity than in the chicken and this smallness of ovalbumin was opposite to the result of CM-cellulose chromatography.

CM-cellulose column chromatography of duck ovalbumin separated by salting-out method—The duck ovalbumin separated by the salting-out method (which was repeated several times for the recrystallization) was followed by the chromatography on CM-cellulose column. As represented in Fig. 3, the diagram shows a major ovalbumin having one sharp peak at *pH* 4.79 and a minor component eluted at a higher *pH* value of 6.30.

Gel filtration of ovalbumin on Sephadex G-100 column—Ovalbumin solution separated by the salting-out method followed with the CM-cellulose column chromatography was sieved on Sephadex G-100 column. The elution diagram is as shown in Fig. 4. The diagram reveals only one sharp peak eluted at the 23th fraction tube and shows the same appearance as observable in case of the sugar of ovalbumin. The eluate peak of chicken ovalbumin was observed at the 27th tube, under the same experimental condition as the case of the duck.

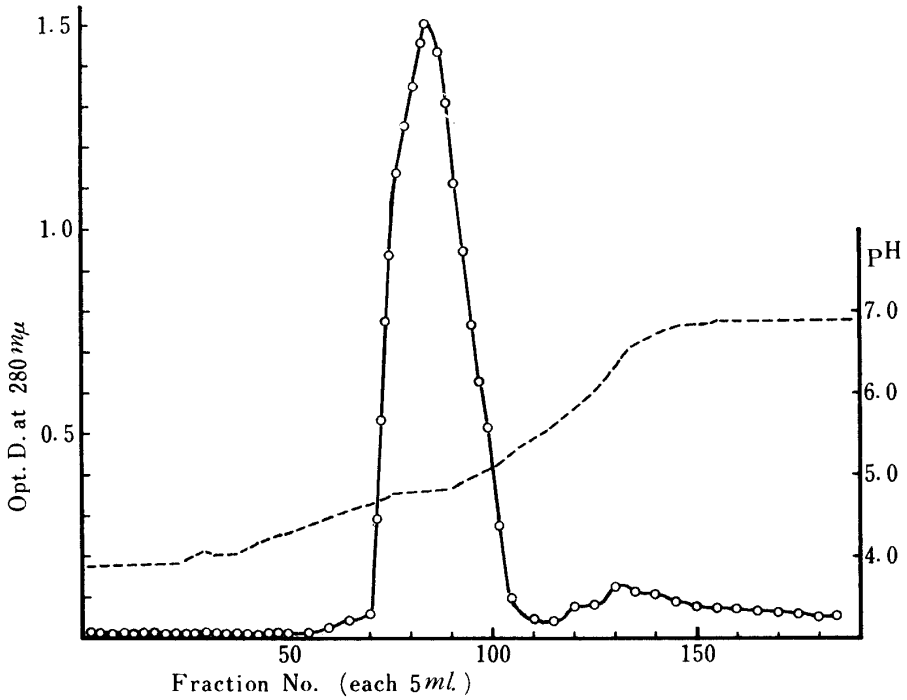

Fig. 3. Chromatography of the duck ovalbumin separated by the salting-out on CM-cellulose column

—○——○— Protein, ——————— *pH*

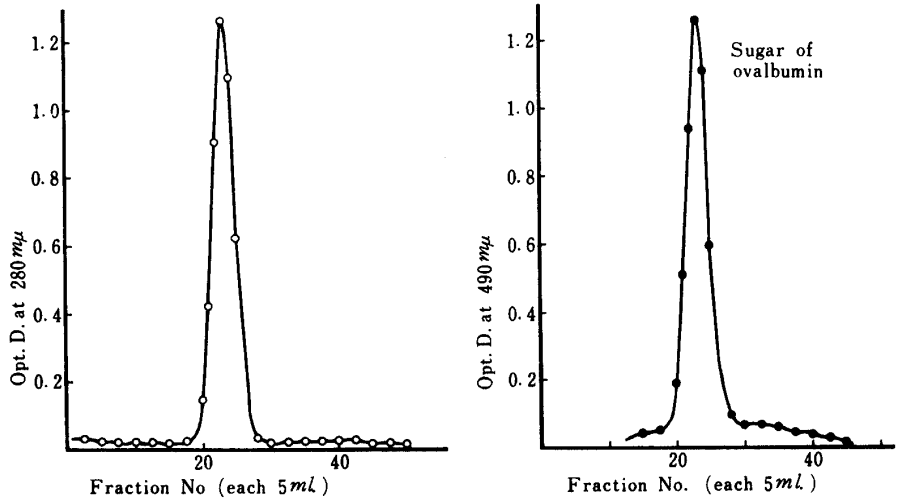

Fig. 4. Gel filtration of the duck ovalbumin refined by the salting-out followed with CMC-chromatography on Sephadex G-100 column

—○——○— Protein, —●——●— Neutral sugar

From these facts, it was ascertained that the molecular weight of duck ovalbumin is slightly larger than the value of chicken ovalbumin (46,000[14]).

On the neutral sugar and hexosamine of ovalbumin——As shown in Fig. 5, ascending chromatography in buthanol-acetic acid-water (12:3:5) gave two distinct spots (Rf values 0.48 and 0.57, respectively) when the solvent was repeatedly run on a given paper in thrice, both of which were capable of reducing the silver nitrate. Rf values of the both spots were respectively agreeing with the authentic galactose and mannose employed together. This chromatograms indicate that duck ovalbumin contains relatively large amount of mannose and small galactose, which differs from the sugar constitution of the chicken ovalbumin.

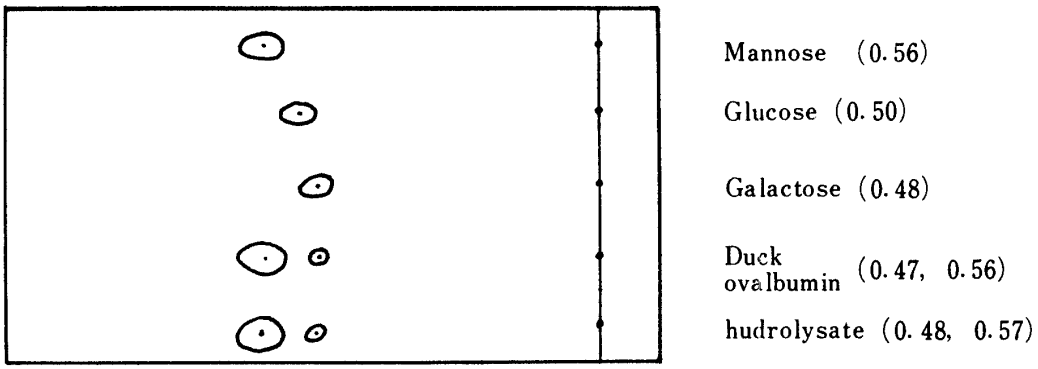

Fig. 5. Paper chromatograms of the hydrolysate of duck ovalbumin and neutral sugars

Ascending chromatography was performed on a given paper in thrice employing BuOH-AcOH-H_2O (12 : 3 : 5, *V/V*) as solvent. Saturated $AgNO_3$-aceton (0.5 : 100, *V/V*) and 0.5N NaOH in EtOH were used for the color development.

Neutral sugar content in duck ovalbumin was found to be amounting to 6.10 %, calculating from the result of Sephadex gel filtration. Sugar content in the ovalbumin separated by the salting-out method was fixed to be coming to 5.92 %, being lower than the former. This result is perhaps due to the contamination of the slight amount of conalbumin containing no sugars. Hexosamine of ovalbumin was identified as glucosamine from the Amberlite CG-120 column chromatography as shown in Fig. 6. The glucosamine content in ovalbumin was ascertained to be amounting to 2.03 %, calculating from the equation of calibration curve $y=15.1 \times 10^{-3}x$ obtained independently concerning the authentic glucosamine, where y is optical density at 530 $m\mu$ and x concentration (*r/ml.*). The molar ratio of neutral sugar and amino sugar was observed to be coming to 3 : 1.

A number of investigations on the carbohydrate of chicken ovalbumin have been published. Johansen, Marshall and Neuberger[15][16] reported 2 % of mannose and 1.2 % of glucosamine (Mol ratio, 5:3) contained in the ovalbumin. After that, determining the mannose by the isotope dilution method, they proposed the content coming to 1.77 %.[17] Lee and Montgomery stated 2.1–2.2 % of mannose and 1.2 % of glucosamine (Mol. ratio, 6:3)[18], Bragg and Hough[19] 2.75 % of mannose and 1.34 % of glucosamine (Mol. ratio, 6.9:3.4). Sugar content in the

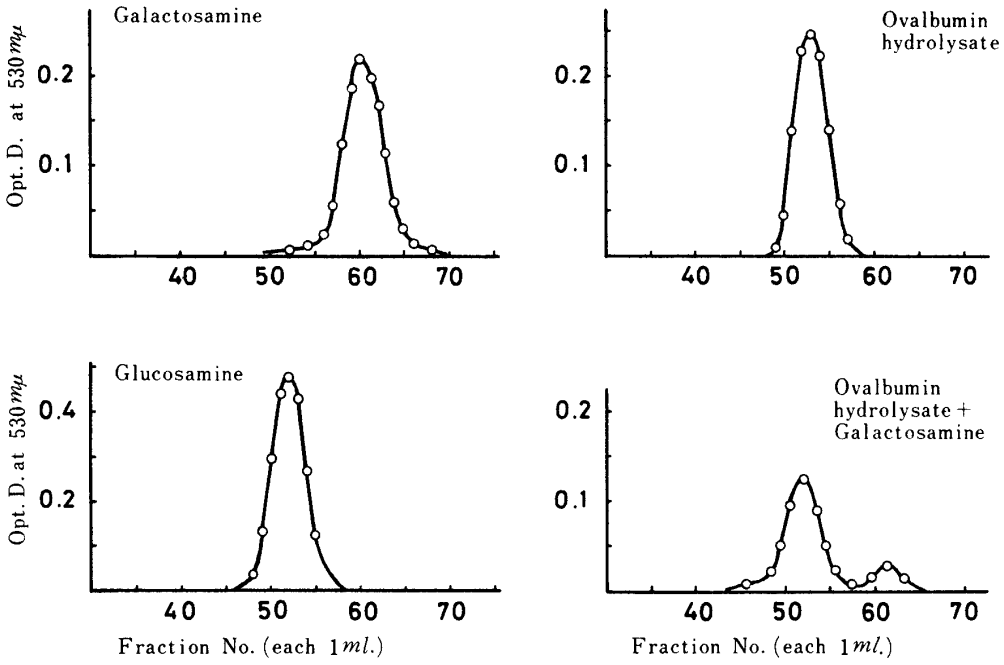

Fig. 6. Chromatography of hexosamines on the Amberlite CG-120 column
Column; 0.6×39 *cm*. Solvent; 0.1*N* HCl Fraction size; 1.0 *ml*.

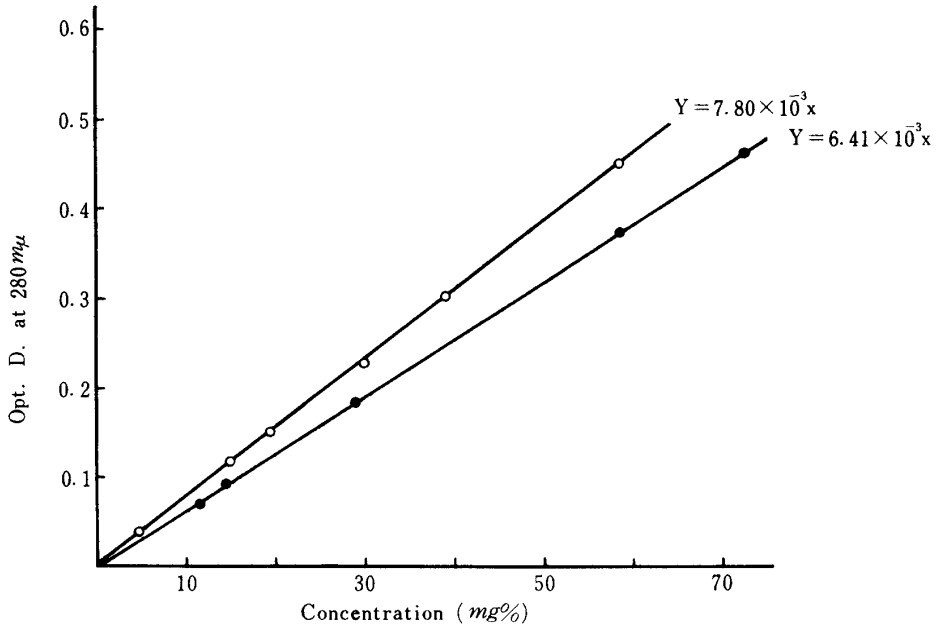

Fig. 7. Calibration curves for duck and chicken ovalbumins
—○—○— Duck —●—●— Chicken

quail ovalbumin including the minor component was ascertained to be coming to 4.3 % by KOGA.[7] Largeness of sugar content in the duck ovalbumin is noteworthy, because such a

large value in ovalbumin has not been found. Largeness of the combined sugar in the whole egg white represented in Table I might due to the largeness of sugar content in ovalbumin and globulin.

The calibration curve for duck ovalbumin—The relation between the absorbance and the concentration of duck ovalbumin compared with the chicken ovalbumin is as shown in Fig. 7. The equation of calibration curve for duck ovalbumin could be represented as $y=7.80\times 10^{-3}x$ and the equation for chicken ovalbumin $y=6.41\times 10^{-3}x$, where y is optical density at 280 $m\mu$ and x *mg %*. These suggest that the duck ovalbumin has a larger amount of aromatic amino acids when compared with the chicken ovalbumin. Before preparing different concentrations of ovalbumin aqueous solution by the diluting treatment, the concentration of original protein solution was gravimetrically determined.

Amino acid composition of ovalbumin—The amino acid compositions of duck ovalbumin calculated from the experiments are presented in Table IV. Those of chicken ovalbumin determined by authors and another worker[14] are shown together in that table. Comparison of the amino acid compositions of duck ovalbumin with those of chicken's one shows that threonine,

Table IV. Amino acid composition of duck ovalbumin compared with chicken ovalbumin
(Values are grams of amino acid residues in 100 *g*. protein. Tryptophan value was calculated from the ultra violet absorption determination. Figures in parentheses were not added in the totals.)

Amino acid	Duck ovalbumin	Chicken ovalbumin	Chicken ovalbumin (by Tristram)[14]
Lysine	4.38	5.30	5.54
Histidine	1.20	1.94	2.08
Ammonia	(1.00)	(0.75)	(1.01)
Arginine	3.78	4.83	5.13
Aspartic acid	6.44	7.49	8.06
Threonine	4.73	3.09	3.81
Serine	6.51	5.91	6.74
Glutamic acid	14.56	13.46	13.24
Proline	3.16	2.96	3.04
Glycine	2.33	2.48	2.32
Alanine	4.58	5.50	5.37
Cystine/2	0.89	1.33	1.58
Valine	5.36	5.56	5.97
Methionine	5.51	4.26	4.60
Isoleucine	4.20	5.77	6.05
Leucine	8.04	6.30	7.95
Tyrosine	3.71	3.38	3.32
Phenylalanine	8.78	6.78	6.81
Tryptophan	1.10	1.10	1.09
Total residues	89.26	87.44	92.70
Neutral sugar	6.10	2.70	1.77[a]
Glucosamine	2.03		1.20[b]

(a) From the references (17)
(b) From the references (16), (18)

glutamic acid, methionine, tyrosine and phenylalanine are more in the duck than in the chicken, while lysine, histidine, arginine, aspartic acid, alanine, cystine and isoleucine in the former is less in quantity than in the latter. Comparing with the chicken ovalbumin, largeness of the total aromatic amino acids in duck coincides with the relation between the both calibration curves shown in Fig. 7. Molar numbers of basic and acidic amino acid residues in both of the ovalbumins are as presented in Table V. Total acidic amino acid residues are superior in molar

Table V. Comparison of basic and acidic amino acid residues in duck and chicken ovalbumins
(Values are molar numbers in 100 g. protein)

Protein / Amino A.	Duck ovalbumin	Chicken ovalbumin
Lysine	34.2×10^{-3}	43.2×10^{-3}
Histidine	$8.8\times$ 〃	$15.2\times$ 〃
Arginine	$24.2\times$ 〃	$32.8\times$ 〃
Total base	$67.2\times$ 〃	$91.2\times$ 〃
Aspartic A.	$56.0\times$ 〃	$70.0\times$ 〃
Glutamic A.	$112.8\times$ 〃	$102.6\times$ 〃
Total acid	$168.8\times$ 〃	$172.6\times$ 〃

numbers to total basic amino acid residues in both of the ovalbumins, the difference amounting to 101.6×10^{-3} *Mol.* in duck, 81.4×10^{-3} *Mol.* in chicken, per 100 *g.* protein, respectively. Presumably these values show that relative acidity is higher in the former than in the latter. And the values are favorable for the relation between eluated *pH* values of both of the ovalbumins on CM-cellulose column chromatography. The aromatic amino acid content is larger in the duck than in the chicken. This relation was agreeing with the descriptions concerning the calibration curves of both of the ovalbumins.

Summary

(1) Between the general chemical properties of duck egg white and those of chicken egg white the following differences were observed. The total- and combined-sugar contents in the former are larger, while specific gravity, nitrogen content, and the specific conductance after dialyzing against the water are smaller than in the latter.

(2) The elution diagram of duck egg white proteins separated by CM-cellulose chromatography revealed eight components. Comparing with the *pH* value of the eluate at each peak of the chicken egg white proteins, values of the duck ovomucoid and ovalbumin are lower respectively. Ovomucoid and ovalbumin are more in quantity in the duck than in the chicken, while the components corresponding to conalbumin and lysozyme in the former are less.

(3) Separation of egg white proteins by the gradient extraction with ammonium sulfate showed seven components in the duck. Comparing with the chicken egg white, in the duck white, ovomucoid and globulin were ascertained to be larger in quantity, globulin being remarkably large. Sugar-protein ratio of the duck globulin was surprisingly high, amounting to 8.50×10^{-2}.

(4) The molecular weight of duck ovalbumin was ascertained to be larger than the value of chicken ovalbumin.
(5) Neutral sugars of duck ovalbumin were fixed to be mannose and galactose, the sugar content amounting to 6.1 %. Hexosamine of ovalbumin was identified as glucosamine, amounting to 2.03 %. Therefore, the molar ratio of neutral sugar and amino sugar was fixed to be 3 : 1.
(6) Comparing with the chicken ovalbumin, in duck ovalbumin, threonine, glutamic acid, methionine, tyrosine and phenylalanine were ascertained to be more, while lysine, histidine, arginine, aspartic acid, alanine, cystine and isoleucine, less in quantity. Acidic amino acid residues were superior in molar numbers to basic amino acid residues in both of the ovalbumins and the difference among those residues being larger in the duck than in the chicken.

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